

Supplementary Materials

Conserved *cis*-regulatory modules in promoters of genes encoding wheat high molecular weight glutenin subunits

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1. Supplementary Figures and Tables

1.1. Supplementary Tables

Supplementary Table 1 Primers and PCR conditions for amplification of the HMW-GS gene promoters.					
Gene	Primer sequence		PCR conditions		
	Forward (5'-3')	Reverse (5'-3')	Annealing temperature (°C) ^a	Elongation time (min)	Fragment size (bp) ^b
<i>Glu-A1-1</i>	GAAGTGTATCGTCTACGGAGGC	GACTACCGCCGCAAAAAGA	65-55 (40)	1	~ 875
<i>Glu-B1-1</i>	CCTATGTTAATTTTAGACATGACTGG	TACTGCCGCAAAGAGGACCAGG	70-60 (40)	1	~ 770
<i>Glu-B1-2</i>	AGCTACCTTCCATTAGTCGG	GAAGATGTTCCCCAAAATATTAC	60 (40)	1	~ 819/ ~ -480
<i>Glu-D1-1</i>	GTTTGGCTAGTTCATTTGTCGTGA	CACTGTAGTTGCTCAGAGGCCT	65-55 (40)	1	~ 662/ ~ -633
<i>Glu-D1-2</i>	ACTGCCGCAAAGAGGACCAG	TGCAACCATGCATCAAAATTC	70-60 (40)	1	~ 1138

^a Interval of temperatures used for touch-down amplification program or the annealing temperature followed by the total number of PCR cycles in brackets.

^b Fragment size followed by the number of nucleotides upstream of the start codon when the reverse primer hybridized downstream of the start codon.

Supplementary Table 2 | List of primers used in this study for gene expression analysis.

Gene	Primer sequence		Fragment size (bp)	Efficiency %
	Forward (5'-3')	Reverse (5'-3')		
<i>Glu-A1-1</i>	CATGCCGACAGGTCGTAG	CTGTTGCGGAGAAGTTACTTA	230	88
<i>Glu-B1-1</i>	GGTGCCGCCCCATCAC	GCAGGTATTCCCCAAAATATCAT	142	87
<i>Glu-B1-2</i>	CCACAAAATAGAGATCAATCACTA	CACGAGGGTGATGACTACTGT	86	87
<i>Glu-D1-1</i>	AGCGGTTAGTCCTCTTTGTGG	CGGAGCTGCTGGTCCATG	157	90
<i>Glu-D1.2</i>	GTTAGCGCAGAGCAGCAAG	CCCTCCATCCGACACACTG	89	93
<i>β-tubulin</i>	CCATCAGTTGGTTGAGAATGC	CAAAGCTGGGAGTGGTCA	101	96
<i>18S RNA</i>	CCATCCCTCCTCCGTAGTTAGCTTCT	CCTGTCTGGCCAAGGCTATATAC	151	93
<i>GAPDH</i>	TTCAACATCATTCCAAGCAGC	CGTAACCCAAAATGCCCTTG	220	92
<i>eF1a</i>	CAGATTGGCAACGGCTACG	CGGACAGCAAAACGACCAAG	227	99

Supplementary Table 3 | DNA oligonucleotides used in EMSA.

Motif name ^a	Sequence (5'-3') ^b
GLM1	atagatgt TGTGAGTCA ttggatag
<i>glm1</i>	atagatgt TtTtAtTa ttggatag
GLM2	atagatat TGTGAGTCA gcatggat
<i>glm2</i>	atagatat TtTtAtTa Agcatgga
G-box	gccca TTACGTGG ctttagcagacc
<i>G-box</i>	gccca TTtctcTGG ctttagcagacc

^a The names of wild-type and mutated motifs are indicated in upper cases and italics, respectively.

^b Bold and uppercase residues correspond to the sequences of the *cis*-motifs GLM1, GLM2 and G-box. Bold and lower case residues indicate mutations in these motifs.

Supplementary Table 4 | DNA accession numbers of HMW-GS gene promoters.

Gene	Promoter haplotype ^a	Accession line No.	GenBank accession No.
<i>Glu-A1-1</i>	h1	748	KM116475
	h2	2135	KM116478
	h3	2358	KM116476
	h4	4482	KM116477
	h5	8048	KM116480
	h6	13812	KM116479
<i>Glu-B1-1^c</i>	h1	2135	KM116484
	h2	964	KM116481
	h3	4901	KM116483
	h4	6529	KM116485
	h5	13310	KM116482
<i>Glu-D1-1^c</i>	h1	748	KM116493
	h2	2135	KM116495
	h3	6086	KM116496
	h4	13812	KM116497
	h5	15658	KM116494
<i>Glu-B1-2</i>	h1	2135	KM116486
	h2	964	KM116487
	h3	8048	KM116488
	h4	5399	KM116489
	h5	13310	KM116490
<i>Glu-D1-2</i>	h1	2135	KM116491
	h2	6086	KM1164927

^aTo avoid redundancy, only one sequence per haplotype (see **Table 1**) was submitted.